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jc184 U.S. PTO 09/610152 07/05/00

UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.	9491-043-27 DIV
First Inventor or Application Identifier	NAPOLEONE FERRARA, ET AL.
Title	IMPROVED ANGIOGENESIS USING HEPATOCYTE GROWTH FACTOR

jc510 U.S. PTO 09/610152 07/05/00

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents

ADDRESS TO: Assistant Commissioner for Patents
Box Patent Application
Washington, DC 20231

1. ■ Fee Transmittal Form (e.g. PTO/SB/17)
(Submit an original and a duplicate for fee processing)

2. ■ Specification — Total Pages: 16

3. □ Drawing(s) *(35 U.S.C. 113)* — Total Sheets:

4. ■ Oath or Declaration — Total Pages: 4
   a. □ Newly executed (original or copy)
   b. ■ Copy from a prior application *(37 C.F.R. §1.63(d))*
   (for continuation/divisional with box 15 completed)
      i. □ DELETION OF INVENTOR(S)
      Signed statement attached deleting inventor(s) named in the prior application, see *37 C.F.R. §1.63(d)(2) and 1.33(b).*

5. ■ Incorporation By Reference *(usable if box 4B is checked)*
The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4B, is considered to be part of the disclosure of the accompanying application and is hereby incorporated by reference therein.

ACCOMPANYING APPLICATION PARTS

6. □ Assignment Papers (cover sheet & document(s))
7. □ 37 C.F.R. §3.73(b) Statement *(when there is an assignee)* □ Power of Attorney
8. □ English Translation Document *(if applicable)*
9. □ Information Disclosure Statement (IDS)/PTO-1449 □ Copies of IDS Citations
10. ■ Preliminary Amendment
11. ■ White Advance Serial No. Postcard
12. □ Small Entity Statement(s) □ Statement filed in prior application. Status still proper and desired.
13. □ Certified Copy of Priority Document(s)
(if foreign priority is claimed)
14. ■ Other: Request For Priority
Revocation (copy executed)

15. If a CONTINUING APPLICATION, *check appropriate box, and supply the requisite information below:*

□ Continuation ■ Divisional □ Continuation-in-part (CIP) of prior application no.: 09/086,498, filed May 22, 1998 (allowed)

Prior application information: Examiner: WEBER, J. Group Art Unit: 1651

16. Amend the specification by inserting before the first line the sentence:

■ This application is a □ Continuation ■ Division □ Continuation-in-part (CIP)

of application Serial No. 09/086,498 Filed on MAY 22, 1998, (allowed), which is a continuation of 08/726,110, filed October 4, 1996, abandoned, which claims benefit of 60/004,816, filed October 5, 1995.

□ This application claims priority of provisional application Serial No. Filed

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Docket No. 9491-043-27 DIV

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF: NAPOLEONE FERRARA, ET AL.
SERIAL NO: NEW DIVISIONAL APPLICATION
FILING DATE: HEREWITH
FOR: IMPROVED ANGIOGENESIS USING HEPATOCYTE GROWTH FACTOR

PRELIMINARY AMENDMENT

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

Prior to the examination on the merits, please enter the following amendments:

IN THE SPECIFICATION:

Page 1, line 9, please delete in its entirety:

"This application is a non-provisional application filed under 37 CFR 1.53 (b) (1) and 35 USC 111 (a), claiming priority under 35 USC 119 (e) to provisional application number 60/004,816 filed October 5, 1995, the contents of which are incorporated herein by reference."

and insert therefor:

- -This application is a divisional of U.S. Application Serial Number 09/086,498, filed May 22, 1998, allowed, which is a continuation of U.S. Application Serial Number 08/726,110, filed October 4, 1996, abandoned, which claims benefit of U.S. Provisional Application Serial

Number 60/004,816, filed October 5, 1995, the contents of all of which are incorporated herein by reference.- -

IN THE CLAIMS:

Please cancel Claims 1-7 without prejudice.

Please add the following new claims:

- -11. A pharmaceutical composition for enhancing angiogenesis comprising hepatocyte growth factor (HGF) in a pharmaceutical carrier acceptable for intravenous, intraarterial or infusion administration.

12. The pharmaceutical composition of Claim 11, wherein said carrier comprises a buffering agent.

13. The pharmaceutical composition of Claim 11, wherein said HGF is recombinant HGF.

14. The pharmaceutical composition of Claim 11, further comprising a pharmacologic agent used to treat conditions associated with vascular disease.

15. The pharmaceutical composition of Claim 14, wherein said pharmacologic agent is vascular endothilial growth factor (VEGF).- -

REMARKS

The specification has been amended to correctly reflect the priority information of the present application. New Claims 11-12 are supported by original non-elected Claims 8-9. Support for new Claim 13 is found at page 10, lines 34-35 and page 11, lines 19-20. New Claims 14 and 15 are supported by page 9, lines 31-34. Claims 8-15 are before the Examiner for consideration.

Applicants believe that this application is now in condition for allowance and therefore request favorable consideration.

If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

Respectfully submitted,

PIPER RUDNICK MARBURY & WOLFE, LLP

07-05-00
Date

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DOCKET NO.: 9491-043-27 DIV

IMPROVED ANGIOGENESIS USING HEPATOCYTE GROWTH FACTOR

Related Applications

This application is a non-provisional application filed under 37 CFR 1.53(b)(1) and 35 USC 111(a), claiming priority under 35 USC 119(e) to provisional application number 60/004,816 filed October 5, 1995, the contents of which are incorporated herein by reference.

Field of the Invention

The invention relates generally to methods and compositions which can be employed for enhancing angiogenesis in mammals.

Background of the Invention

Hepatocyte growth factor ("HGF") functions as a growth factor for particular tissues and cell types. HGF was identified initially as a mitogen for hepatocytes [Michalopoulos et al., Cancer Res., 44:4414-4419 (1984); Russel et al., J. Cell. Physiol., 119:183-192 (1984); Nakamura et al., Biochem. Biophys. Res. Comm., 122:1450-1459 (1984)]. Nakamura et al., supra, reported the purification of HGF from the serum of partially hepatectomized rats. Subsequently, HGF was purified from rat platelets, and its subunit structure was determined [Nakamura et al., Proc. Natl. Acad. Sci. USA, 83:6489-6493 (1986); Nakamura et al., FEBS Letters, 224:311-316 (1987)]. The purification of human HGF ("huHGF") from human plasma was first described by Gohda et al., J. Clin. Invest., 81:414-419 (1988).

Both rat HGF and huHGF have been molecularly

cloned, including the cloning and sequencing of a naturally occurring variant lacking 5 amino acids designated "delta5 HGF" [Miyazawa et al., Biochem. Biophys. Res. Comm., 163:967-973 (1989); Nakamura et al., Nature, 342:440-443 (1989); Seki et al, Biochem. Biophys. Res. Commun., 172:321-327 (1990); Tashiro et al., Proc. Natl. Acad. Sci. USA, 87:3200-3204 (1990); Okajima et al., Eur. J. Biochem., 193:375-381 (1990)].

The mature form of huHGF, corresponding to the major form purified from human serum, is a disulfide linked heterodimer derived by proteolytic cleavage of the human pro-hormone between amino acids R494 and V495. This cleavage process generates a molecule composed of an α-subunit of 440 amino acids (M_r 69 kDa) and a β-subunit of 234 amino acids (M_r 34 kDa). The nucleotide sequence of the huHGF cDNA reveals that both the α- and the β-chains are contained in a single open reading frame coding for a pre-pro precursor protein. In the predicted primary structure of mature huHGF, an interchain S-S bridge is formed between Cys 487 of the α-chain and Cys 604 in the β-chain [see Nakamura et al., Nature, supra]. The N-terminus of the α-chain is preceded by 54 amino acids, starting with a methionine group. This segment includes a characteristic hydrophobic leader (signal) sequence of 31 residues and the prosequence. The α-chain starts at amino acid (aa) 55, and contains four kringle domains. The kringle 1 domain extends from about aa 128 to about aa 206, the kringle 2 domain is between about aa 211 and about aa 288, the kringle 3 domain is defined as extending from about aa 303 to about aa 383, and the kringle 4 domain extends from about aa 391 to about aa 464 of the α-chain.

The definition of the various kringle domains is based on their homology with kringle-like domains of other proteins (such as prothrombin and plasminogen), therefore, the above limits are only approximate. To date, the function of these kringles has not been determined. The β-chain of huHGF shows high homology to the catalytic domain of serine

proteases (38% homology to the plasminogen serine protease domain). However, two of the three residues which form the catalytic triad of serine proteases are not conserved in huHGF. Therefore, despite its serine protease-like domain, huHGF appears to have no proteolytic activity, and the precise role of the β-chain remains unknown. HGF contains four putative glycosylation sites, which are located at positions 294 and 402 of the α-chain and at positions 566 and 653 of the β-chain.

In a portion of cDNA isolated from human leukocytes, in-frame deletion of 15 base pairs was observed. Transient expression of the cDNA sequence in COS-1 cells revealed that the encoded HGF molecule (delta5 HGF) lacking 5 amino acids in the kringle 1 domain was fully functional [Seki et al., supra].

A naturally occurring huHGF variant has been identified which corresponds to an alternative spliced form of the huHGF transcript containing the coding sequences for the N-terminal finger and first two kringle domains of mature huHGF [Chan et al., Science, 254:1382-1385 (1991); Miyazawa et al., Eur. J. Biochem., 197:15-22 (1991)]. This variant, designated HGF/NK2, has been proposed to be a competitive antagonist of mature huHGF.

Comparisons of the amino acid sequence of rat HGF with that of huHGF have revealed that the two sequences are highly conserved and have the same characteristic structural features. The length of the four kringle domains in rat HGF is exactly the same as in huHGF. Furthermore, the cysteine residues are located in exactly the same positions, an indication of similar three-dimensional structures [Okajima et al., supra; Tashiro et al., supra].

HGF and HGF variants are described further in U.S. Patent Nos. 5,227,158, 5,316,921, and 5,328,837.

The HGF receptor has been identified as the product of the c-Met proto-oncogene [Bottaro et al., Science,

251:802-804 (1991); Naldini et al., Oncogene, 6:501-504 (1991); WO 92/13097 published August 6, 1992; WO 93/15754 published August 19, 1993]. The receptor is usually referred to as "c-Met" or "p190^{MET}" and typically comprises, in its native form, a 190-kDa heterodimeric (a disulfide-linked 50-kDa α-chain and a 145-kDa β-chain) membrane-spanning tyrosine kinase protein [Park et al., Proc. Natl. Acad. Sci. USA, 84:6379-6383 (1987)]. Several truncated forms of the c-Met receptor have also been described [WO 92/20792; Prat et al., Mol. Cell. Biol., 11:5954-5962 (1991)].

The binding activity of HGF to its receptor is believed to be conveyed by a functional domain located in the N-terminal portion of the HGF molecule, including the first two kringles [Matsumoto et al., Biochem. Biophys. Res. Commun., 181:691-699 (1991); Hartmann et al., Proc. Natl. Acad. Sci., 89:11574-11578 (1992); Lokker et al., EMBO J., 11:2503-2510 (1992); Lokker and Godowski, J. Biol. Chem., 268:17145-17150 (1991)]. The c-Met protein becomes phosphorylated on tyrosine residues of the 145-kDa β-subunit upon HGF binding.

Various biological activities have been described for HGF and its receptor [see, generally, Chan et al., Hepatocyte Growth Factor-Scatter Factor (HGF-SF) and the C-Met Receptor, Goldberg and Rosen, eds., Birkhauser Verlag-Basel (1993), pp. 67-79]. It has been observed that levels of HGF increase in the plasma of patients with hepatic failure [Gohda et al., supra] and in the plasma [Lindroos et al., Hepatol., 13:734-750 (1991)] or serum [Asami et al., J. Biochem., 109:8-13 (1991)] of animals with experimentally induced liver damage. The kinetics of this response are usually rapid, and precedes the first round of DNA synthesis during liver regeneration. HGF has also been shown to be a mitogen for certain cell types, including melanocytes, renal tubular cells, keratinocytes, certain endothelial cells and cells of epithelial origin [Matsumoto et al., Biochem. Biophys. Res.

Commun., 176:45-51 (1991); Igawa et al., Biochem. Biophys. Res. Commun., 174:831-838 (1991); Han et al., Biochem., 30:9768-9780 (1991); Rubin et al., Proc. Natl. Acad. Sci. USA, 88:415-419 (1991)]. Both HGF and the c-Met protooncogene have been postulated to play a role in microglial reactions to CNS injuries [DiRenzo et al., Oncogene, 8:219-222 (1993)].

HGF can also act as a "scatter factor", an activity that promotes the dissociation of epithelial and vascular endothelial cells *in vitro* [Stoker et al., Nature, 327:239-242 (1987); Weidner et al., J. Cell Biol., 111:2097-2108 (1990); Naldini et al., EMBO J., 10:2867-2878 (1991); Giordano et al., Proc. Natl. Acad. Sci. USA, 90:649-653 (1993)]. Moreover, HGF has recently been described as an epithelial morphogen [Montesano et al., Cell, 67:901-908 (1991)]. Therefore, HGF has been postulated to be important in tumor invasion [Comoglio, Hepatocyte Growth Factor-Scatter Factor (HGF-SF) and the C-Met Receptor, Goldberg and Rosen, eds., Birkhauser Verlag-Basel (1993), pp. 131-165].

Therapeutic options for patients with vascular disease, particularly vascular obstructive disease, are sometimes limited. As Takeshita et al., J. Clin. Invest., 93:662-670 (1994), point out, such patients are often refractory to conservative measures and typically unresponsive to drug therapy. When vascular obstruction is lengthy and/or widespread, nonsurgical revascularization may not be feasible. Id. Surgical therapy, consisting of arterial bypass and/or amputation, may be complicated by a variable morbidity and mortality, and is often dependent for its efficacy upon short- and long-term patency of the conduit used. Id. Therapeutic angiogenesis thus constitutes an alternative treatment strategy for such patients.

Summary of the Invention

The invention provides methods for enhancing angiogenesis in a mammal comprising administering to the

mammal an effective amount of HGF. The HGF alone may be administered to the mammal, or alternatively, may be administered to the mammal in combination with other therapies and/or pharmacologic agents.

The invention also provides articles of manufacture and kits which contain HGF.

Although not being bound by any particular theory, it is presently believed that the HGF can be used to stimulate or enhance angiogenic activity in patients suffering from vascular insufficiency or limb ischemia secondary to arterial occlusive disease.

Detailed Description of the Invention

I. Definitions

As used herein, the terms "hepatocyte growth factor" and "HGF" refer to a growth factor typically having a structure with six domains (finger, Kringle 1, Kringle 2, Kringle 3, Kringle 4 and serine protease domains). Fragments of HGF constitute HGF with fewer domains and variants of HGF may have some of the domains of HGF repeated; both are included if they still retain their respective ability to bind a HGF receptor. The terms "hepatocyte growth factor" and "HGF" include hepatocyte growth factor from humans ("huHGF") and any non-human mammalian species, and in particular rat HGF. The terms as used herein include mature, pre, pre-pro, and pro forms, purified from a natural source, chemically synthesized or recombinantly produced. Human HGF is encoded by the cDNA sequence published by Miyazawa et al., 1989, supra, or Nakamura et al., 1989, supra. The sequences reported by Miyazawa et al. and Nakamura et al. differ in 14 amino acids. The reason for the differences is not entirely clear; polymorphism or cloning artifacts are among the possibilities. Both sequences are specifically encompassed by the foregoing terms. It will be understood that natural allelic variations exist and can occur among individuals, as

demonstrated by one or more amino acid differences in the amino acid sequence of each individual. The HGF of the invention preferably has at least about 80% sequence identity, more preferably at least about 90% sequence identity, and even more preferably, at least about 95% sequence identity with a native mammalian HGF. The terms "hepatocyte growth factor" and "HGF" specifically include the delta5 huHGF as disclosed by Seki et al., supra.

The terms "HGF receptor" and "c-Met" when used herein refer to a cellular receptor for HGF, which typically includes an extracellular domain, a transmembrane domain and an intracellular domain, as well as variants and fragments thereof which retain the ability to bind HGF. The terms "HGF receptor" and "c-Met" include the polypeptide molecule that comprises the full-length, native amino acid sequence encoded by the gene variously known as $p190^{MET}$. The present definition specifically encompasses soluble forms of HGF receptor, and HGF receptor from natural sources, synthetically produced *in vitro* or obtained by genetic manipulation including methods of recombinant DNA technology. The HGF receptor variants or fragments preferably share at least about 65% sequence homology, and more preferably at least about 75% sequence homology with any domain of the human c-Met amino acid sequence published in Rodrigues et al., Mol. Cell. Biol., 11:2962-2970 (1991); Park et al., Proc. Natl. Acad. Sci., 84:6379-6383 (1987); or Ponzetto et al., Oncogene, 6:553-559 (1991).

The term "angiogenesis" is used herein in a broad sense and refers to the production or development of blood vessels.

The terms "treating," "treatment," and "therapy" as used herein refer to curative therapy, prophylactic therapy, and preventative therapy.

The term "mammal" as used herein refers to any mammal classified as a mammal, including humans, cows, horses,

dogs and cats. In a preferred embodiment of the invention, the mammal is a human.

II. Compositions and Methods of the Invention

The present invention provides methods for enhancing angiogenesis using hepatocyte growth factor, referred to hereinafter as "HGF". The HGF useful in the practice of the present invention can be prepared in a number of ways. For instance, the HGF can be prepared using an isolated or purified form of HGF. Methods of isolating and purifying HGF from natural sources are known in the art. Such isolation and purification methods can be employed for obtaining HGF from serum or plasma. Alternatively, HGF can be chemically synthesized and prepared using recombinant DNA techniques known in the art and described in further detail the Example below.

The HGF may be from human or any non-human species. For instance, a mammal may have administered HGF from a different mammalian species (e.g., rats can be treated with human HGF). Preferably, however, the mammal is treated with homologous HGF (e.g., humans are treated with human HGF) to avoid potential immune reactions to the HGF. The HGF is typically administered to a mammal diagnosed as having some form of vascular insufficiency or vascular disease. It is of course contemplated that the methods of the invention can be employed in combination with other therapeutic techniques such as surgery.

The HGF is preferably administered to the mammal in a pharmaceutically-acceptable carrier. Suitable carriers and their formulations are described in Remington's Pharmaceutical Sciences, 16th ed., 1980, Mack Publishing Co., edited by Oslo et al. Typically, an appropriate amount of a pharmaceutically-acceptable salt is used in the formulation to render the formulation isotonic. Examples of the pharmaceutically-acceptable carrier include liquids such as

saline, Ringer's solution and dextrose solution. The pH of the solution is preferably from about 5 to about 8, and more preferably from about 7 to about 7.5. The formulation may also comprise a lyophilized powder. Further carriers include sustained release preparations such as semipermeable matrices of solid hydrophobic polymers, which matrices are in the form of shaped articles, e.g., films, liposomes or microparticles. It will be apparent to those persons skilled in the art that certain carriers may be more preferable depending upon, for instance, the route of administration and concentration of HGF being administered.

The HGF can be administered to the mammal by injection (e.g. intravenous, intraarterial, intraperitoneal, subcutaneous, intramuscular), or by other methods such as infusion that ensure its delivery to the bloodstream in an effective form. Optionally, the HGF may be administered by direct intraarterial administration upstream from an occluded artery to optimize concentration and activity of HGF in the local circulation of an affected limb.

Effective dosages and schedules for administering the HGF may be determined empirically, and making such determinations is within the skill in the art. Those skilled in the art will understand that the dosage of HGF that must be administered will vary depending on, for example, the mammal which will receive the HGF, the route of administration, the particular type of HGF used and other drugs being administered to the mammal. A typical daily dosage of the HGF used alone might range from about 1 μg/kg to up to 100 mg/kg of body weight or more per day, depending on the factors mentioned above.

HGF may also be administered along with other pharmacologic agents used to treat the conditions associated with vascular disease such as vascular endothelial growth factor ("VEGF"). The HGF may be administered sequentially or concurrently with the one or more other pharmacologic agents.

The amounts of HGF and pharmacologic agent depend, for example, on what type of drugs are used, the specific condition being treated, and the scheduling and routes of administration.

Following administration of HGF to the mammal, the mammal's physiological condition can be monitored in various ways well known to the skilled practitioner.

In another embodiment of the invention, there are provided articles of manufacture and kits containing materials useful for enhancing angiogenesis. The article of manufacture comprises a container with a label. Suitable containers include, for example, bottles, vials, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is effective for enhancing angiogenesis. The active agent in the composition is HGF. The label on the container indicates that the composition is used for enhancing angiogenesis, and may also indicate directions for *in vivo* use, such as those described above.

The kit of the invention comprises the container described above and a second container comprising a pharmaceutically-acceptable buffer, such as phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

The invention will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of the invention. All reference citations herein are incorporated by reference.

Example

Recombinant human HGF ("rhuHGF") was produced in CHO cells using a procedure modified from Naka et al., J.

Biol. Chem., 267:20114-20119 (1992). rhuHGF-transfected cells were grown in a 400 L bioreactor in medium containing 2% fetal bovine serum for 8 days. Culture supernatant containing rhuHGF was concentrated and clarified, then conditioned by the addition of solid NaCl to 0.3 M. rhuHGF was then purified in a single step using cation exchange chromatography. Conditioned, concentrated culture supernatant was loaded onto a column of S-Sepharose Fast Flow equilibrated in 20 mM Tris, pH 7.5, 0.3 M NaCl. After washing out unbound protein, rhuHGF was eluted in a linear gradient from 20 mM Tris, pH 7.5, 0.3 M NaCl to 20 mM Tris, pH 7.5, 1.2 M NaCl. rhuHGF-containing fractions were pooled based on SDS-PAGE analysis. The S Sepharose Fast Flow pool was concentrated and exchanged into 20 mM Tris, pH 7.5, 0.5 M NaCl by gel filtration on Sephadex G25 to a final concentration of about 3-5 mg/ml. A rhuHGF stock solution was then prepared by diluting the rhuHGF in buffer (0.5% bovine serum albumin, 0.05% Tween-20, 0.01% Thimersol in PBS).

The effects of rhuHGF on angiogenesis was tested in a rabbit model of hindlimb ischemia. The rabbit model was designed to simulate ischemia characteristics of patients with severe lower extremity arterial occlusive disease. [Takeshita et al., supra]. The effects of vascular endothelial growth factor ("VEGF") were also tested and compared to rhuHGF. The *in vivo* experiment was conducted essentially as described in Takeshita et al., supra. One femoral artery was resected in each of 24 New Zealand rabbits. Ten days later (Day 0 of study), baseline measurements of calf blood pressure (BP) index; angiographic score of collateral formation; intravascular Doppler-wire analysis of blood flow; and microsphere-based analysis of muscle perfusion at rest and during stress were performed. The animals exhibited similar baseline measurements.

Each group of animals (8 rabbits/group) then received intra-iliac rhuHGF (500 μg), recombinant human VEGF

("rhuVEGF") (500 μg) [prepared as described in Ferrara et al., Methods Enzym., 198:391-404 (1991)], or vehicle (saline plus 0.1% rabbit serum albumin), followed by the same dose intravenously at Days 2 and 4 of the study. At Day 30, all measurements were repeated, and the animals were sacrificed. Total muscle weight of each leg was measured and samples were used for capillary density. The results at Day 30 are shown below in Table 1.

TABLE 1

Day 30 Data	Vehicle	rhuVEGF	rhuHGF
Angiographic Score	0.46 ± 0.06	0.62 ± 0.04†	0.78 ± 0.07†§
Capillary Density (/mm2)	158 ± 12	247 ± 18†	282 ± 15†§
BP index (%)	51.6 ± 4.5	69.8 ± 3.1†	84.5 ± 1.8†§
Blood flow (ml/min)	17.9 ± 1.1	20.6 ± 1.3*	23.4 ± 1.2†§
Muscle perfusion (rest, %)	73.2 ± 6.8	88.4 ± 6.6*	99.2 ± 4.5†§
Muscle perfusion (stress, %)	36.6 ± 8.8	65.7 ± 7.5†	83.3 ± 6.7†§
Muscle weight (%)	73.0 ± 2.6	87.6 ± 2.8*	95.9 ± 5.4†§

% = % of normal limb; * = p<.05 vs vehicle; † = p<.001 vs vehicle; § = p<.05 vs VEGF

The data showed that HGF enhanced collateral vessel formation and regional perfusion, and prevented atrophy. At similar doses in the study, the HGF exhibited

greater efficiency than VEGF.

What is claimed is:

1. A method of enhancing angiogenesis in a mammal, comprising administering an effective amount of hepatocyte growth factor ("HGF") to the mammal.

2. The method of claim 1 wherein said HGF has at least 80% sequence identity with a native mammalian HGF.

3. The method of claim 2 wherein said HGF has at least 90% sequence identity with a native mammalian HGF.

4. The method of claim 3 wherein said HGF has at least 95% sequence identity with a native mammalian HGF.

5. The method of claim 4 wherein said HGF is human HGF.

6. The method of claim 1 wherein said HGF is administered intraarterially.

7. The method of claim 6 wherein HGF is additionally administered intravenously.

8. An article of manufacture, comprising:

a container;

a label on said container; and

a composition comprising an active agent contained within said container;

wherein the composition is effective for enhancing angiogenesis, the label on said container indicates that the composition can be used for enhancing angiogenesis, and the active agent in said composition comprises HGF.

9. The article of manufacture of claim 8 further comprising instructions for administering the HGF to a mammal to enhance angiogenesis.

10. A kit, comprising:

a first container, a label on said container, and a composition comprising an active agent contained within said container;

wherein the composition is effective for enhancing angiogenesis, the label on said container indicates that the composition can be used for enhancing angiogenesis, and the active agent in said composition comprises HGF;

a second container comprising a pharmaceutically-acceptable buffer; and

instructions for using the HGF to enhance angiogenesis.

Abstract of the Disclosure

Methods for enhancing angiogenesis in a mammal using hepatocyte growth factor ("HGF") are provided. In the methods, HGF can be administered to mammals suffering from, for instance, vascular insufficiency or arterial occlusive disease. Articles of manufacture and kits containing HGF are also provided.

Docket No. P0972R1

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY

COPY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

IMPROVED ANGIOGENESIS USING HEPATOCYTE GROWTH FACTOR

the specification of which (check one) _ is attached hereto or X was filed on October 4, 1996 as Application Serial No. 08/726,110 and was amended on (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate have a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s):			Priority Claimed	
			Yes	No
Number	Country	Day/Month/Year Filed		

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional applications(s) listed below:

60/004,816	October 5, 1995
Application Ser. No.	Filing Date

I hereby claim the benefit under Title 35, United States Code, §120 of any United States applications(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application Ser. No.	Filing Date	Status: Patented, Pending, Abandoned
Application Ser. No.	Filing Date	Status: Patented, Pending, Abandoned

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

Walter E. Buting - Reg. No. 23,092
Deirdre L. Conley - Reg. No. 36,487
Ginger R. Dreger - Reg. No. 33,055
Janet E. Hasak - Reg. No. 28,616
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Wendy M. Lee - Reg. No. 40,378
Richard B. Love - Reg. No. 34,659
Diane L. Marschang - Reg. No. 35,600
Craig G. Svoboda - Reg. No. 39,044
Timothy E. Torchia - Reg. No. 36,700
Daryl B. Winter - Reg. No. 32,637

Send correspondence to Genentech, Inc.
Attn: Diane L. Marschang
460 Point San Bruno Boulevard
South San Francisco, CA 94080
Telephone: (415) 225-5416

I hereby declare that all statements made herein of my own knowledge and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that willful false statements may jeopardize the validity of the application or any patent issued thereon.

☐ The undersigned hereby authorizes the U.S. attorney or agent named herein to accept and follow instructions from his foreign patent agent as to any action to be taken in the Patent and Trademark Office regarding this application without direct communication between the U.S. attorney or agent and the undersigned. In the event of a change in the persons from whom instructions may be taken, the U.S. attorney or agent named herein will be so notified by the undersigned.

Full name of sole or first inventor

Napoleone Ferrara

Napoleone Ferrara — 1-25-93

Inventor's signature — Date

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Full name of third joint inventor, if any

Ralph H. Schwall

Inventor's signature

Ralph Schwall

Date

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF: NEPOLEONE FERRARA, ET AL. GAU: 1651

SERIAL NO: 09/086,498 EXAMINER: WEBER, J.

FILING DATE: MAY 22, 1998

FOR: IMPROVED ANGIOGENESIS USING HEPATOCYTE GROWTH FACTOR

REVOCATION AND NEW APPOINTMENT OF POWER OF ATTORNEY

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

The undersigned representative of GENENTECH, INC., owner by virtue of assignment of the above-identified application, hereby revokes any and all previous Powers of Attorney and appoints Steven B. Kelber, Reg. No. 30,073; Marc R. Labgold, Ph.D., Reg. No. 34,651; Sharon E. Crane, Ph.D., Reg. No. 36,113; Catherine Bax Richardson, Reg. No. 39,007 and Ron Myers, M.S., Reg. No. 43,825 as Assignee's attorney with full power of substitution and revocation, to prosecute said patent application, receive any Letters Patent and to take any and all other actions with regard to this patent application and any Letters Patent issuing thereon, and request that all correspondence be sent to Steven B. Kelber of LONG ALDRIDGE & NORMAN, LLP whose post office address is: 701 Pennsylvania Avenue, N.W., 6th Floor, Washington, D.C. 20004.

CERTIFICATION UNDER 37 C.F.R. 3.73(b)

I, the undersigned, certify that I am an individual empowered to act on behalf of GENENTECH, INC. the assignee of the entire right, title and interest of the above-identified application by virtue of an assignment from the inventor(s)

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

04/09/99
Date Signed

Sean Johnston TRS
Person Signing Sean Johnston

V.P. Intellectual Property
Signor's Title

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

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Ginger R. Dreger - Reg. No. 33,055
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Full name of sole or first inventor
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